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generation and network formation. In contrast, exposing the cells to Bacterial SMase C to hydrolyze of sphingomyelin didn't affect neither endothelial biomechanics not morphogenesis indicating that OxLDL-induced stimulation of SMAse cannot be responsible for these effects.

1617-Pos Board B461

A Zipper Network Model of Extracellular Matrix Failure Reveals a New Role for Proteoglycans

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Mechanical failure of soft tissues is characteristic of life threatening diseases, including emphysema and vessel wall aneurysms. Failure occurs when mechanical forces are sufficient to rupture the enzymatically weakened extracellular matrix (ECM). Elastin is an important structural protein of the ECM, and is known to stretch beyond 200% strain before failing. However, ECM constructs and native vessel walls composed primarily of elastin and proteoglycans (PGs) have been found to fail at much lower strains. In this study, we hypothesized that PGs significantly contribute to tissue failure. To test this, we developed a novel Zipper Network Model (ZNM), in which springs representing elastin are organized into long wavy fibers in a zipper-like formation and placed within a network of springs mimicking PGs. Elastin and PG springs possessed distinct mechanical and failure properties. The elastin does not percolate while the PGs can serve as bridges between elastin fibers as well as hinder folding of the fibers via bond-bending. During stretching, elastin fibers first become straight, then start stretching the PG bridges. Simulations using the ZNM showed that the failure of PGs alone reduces the global failure strain of the ECM well below that of elastin and hence digestion of elastin does not influence the failure strain. Network analysis also suggested that elastin determines the peak and failure stress while PGs transmit the load and define the failure strain of the network. Predictions of the ZNM were experimentally confirmed by measuring the failure properties of engineered ECM constructs before and after digestion with trypsin that cleaves the core protein of PGs without affecting elastin. This study reveals a novel role for PGs in the failure mechanics of engineered and native ECM with implications for the design of engineered tissues.

1618-Pos Board B462

Biophysical Regulation of Endoderm By 3-Dimensional Fibronectin Matrix

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Fibronectin (FN), a major extracellular matrix (ECM) component that assembles into a 3-dimensional (3D) network, plays a significant role in the development and maintenance of most tissues. In the embryonic stem (ES) cell niche, ECM composition, elasticity, and architecture likely contribute to the decision between self-renewal and differentiation. ES cells differentiating as multicellular embryoid bodies (EBs) exhibit a 10-fold drop in expression of Nanog, a self-renewal marker, concurrent with a 3-fold upregulation in FN production as well as the onset of differentiation markers Fgf 5 (ectoderm), brachyury (mesoderm), and GATA4 (endoderm). However, FN and GATA4 appear to be temporally and spatially correlated within the EB while FN and Nanog are inversely correlated with each other. To probe any specific FN-GATA4 interaction and its biophysical regulation, FN-coated surfaces and 3-dimensional, soft fibrillar FN matrices were used as substrates for ES cells grown in monolayer culture. ES cells on FN-coated surfaces displayed a well spread morphology but did not significantly increase their FN production or GATA4 expression. In contrast, ES cells grown on fibrillar matrices were less spread, displayed a 4-fold upregulation of FN production similar to that of EBs, and expressed GATA4 via immunofluorescent detection. However, when crosslinked to increase 3D FN matrix elasticity from 350 Pa to 4500 Pa, FN expression dropped 2-fold and GATA4 staining was significantly reduced. Though the specific molecular mechanisms require elucidation, these findings suggest important temporal, spatial, and mechanical roles for FN matrix in regulation of ES cell development.

1619-Pos Board B463

Dynamic Behavior Of Heterogeneous Cell Populations Growing Under Mass Transport Limitations

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Tissue growth in biomimetic scaffolds is strongly influenced by the dynamics and heterogeneity of cell populations. A significant source of heterogeneity is nutrient (or growth factor) depletion. Cells slow down, stop dividing or die when the concentrations of key nutrients or growth factors drop below critical levels in the scaffold interior. As a result, we still cannot grow *in vitro* tissue samples thicker than a few millimeters for metabolically active cells.
To provide theoretical guidance for the *in vitro* cultivation of bioartificial tissues, we have developed a multi-scale model that can describe how the complex interplay among key intracellular processes, cell population dynamics and nutrient depletion regulates the growth of tissues in 3D scaffolds. We use a discrete, stochastic algorithm to describe the population dynamics of migrating, interacting and proliferating cells. Diffusion and consumption of a limiting nutrient is modeled by a partial differential equation subject to boundary conditions appropriate for common bioreactors. This PDE is solved numerically and the computed concentration profiles are used to modulate cell proliferation rates and migration speeds. The hybrid discrete-continuous model was parallelized and solved on a distributed-memory multicomputer to study how mass transport limitations affect tissue regeneration rates under conditions encountered in typical bioreactors.
Simulation results show that the severity of mass transport limitations can be estimated by the magnitude of two dimensionless groups. Critical system parameters like cell population heterogeneity, the initial spatial distribution of seed cells, the distribution of cell migration speeds, and the hydrodynamic environment are shown to affect not only the overall rate, but also the pattern of tissue growth. More specifically, the interplay of cell population heterogeneity and cell death due to nutrient depletion can lead to dynamic self-assembly of cells and the formation of stratified structures.

1620-Pos Board B464

Velocity-dependence of Cargo Loading onto Molecular Shuttles Demonstrates the Glue-like Character of Biotin/Streptavidin

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Molecular shuttles based on biomolecular motors and their associated filaments are being developed to function as conveyor belts in the molecular factories of the future. An essential design element in these active nanoscale transport systems is cargo loading onto the shuttles. We demonstrate that molecular shuttle velocity has to be optimized to facilitate cargo attachment of nanospheres via biotin-streptavidin linkages. The biotin-streptavidin bond gains its ultimate strength on a timescale of milliseconds due to existence of metastable binding states. As a consequence of the glue-like character of this widely used intermolecular bond, the velocity of molecular shuttles has to be optimized to permit efficient attachment of cargo via biotin-streptavidin linkages.
In our experiments, kinesin motor proteins adsorbed to a casein precoated surface were used to propel biotinylated microtubules which were coated with streptavidin at saturating dosages. The microtubule gliding velocity was varied between 50 nm/s and 450 nm/s by changing the kinesin substrate ATP concentration. Finally, biotinylated fluorescein-labeled nanospheres were added in concentrations ranging from 25 pM to 100 pM. Nanospheres attached to the surface and were loaded onto microtubules only as a result of collisions between gliding microtubules and nanospheres. Nanosphere attachment showed an unexpected optimum at an intermediate shuttle velocity.
The attachment and detachment processes were modeled by combining rigorous mechanical engineering analysis with detailed physico-chemical models. This contribution will present both, the experimental details of our velocity dependent loading experiments and the theoretical model which explains the optimum on the basis of the complex binding energy landscape of the biotin streptavidin linkages.

1621-Pos Board B465

Correlation Between Antibody Affinity and Activity: Understanding the Molecular Basis for a picomolar to femtomolar Increase in Affinity

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A chimeric antibody was human adapted and then affinity matured. Biological activity studies revealed that the affinity matured antibody is 10-fold more potent that the chimeric antibody. To determine the correlation between affinity and activity of these antibodies, the binding profile to their antigen was analyzed by Biacore and Kinexa. The studies showed the two mAbs have different thermodynamic profiles. These differences, particularly the equilibrium dissociation constant, K_D, revealed a positive correlation with potency (biological activity). The data showed that the affinity of the chimeric antibody is picomolar, whereas the affinity of the human adapted antibody is femtomolar. Molecular modeling studies showed that several of the mutations introduced in the CDRs during the affinity maturation process were hydrophobic replacements

of hydrophilic amino acids. The replacements are mostly located at the periphery of the antigen binding-site but are potentially in contact with the antigen, thus encircling the center of the epitope recognized by these antibodies. These hydrophobic contacts may account for more than 15- and 10-fold increase in affinity and potency, respectively.

Intrinsically Disordered Proteins

1622-Pos Board B466

Protein Folding as a Transition Step from Ancient to the Modern Life Forms

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Several lines of evidence suggest that the first proteins on earth likely contained significantly fewer than the modern 20 amino acids. Subsequently many researchers examined the evolution of the genetic code using different criteria. Trifonov combined 40 different of these single-factor criteria into a consensus and proposed the following temporal order of addition for the amino acids: G/A, V/D, P, S, E/L, T, R, N, K, Q, I, C, H, F, M, Y, W. Brooks and co-workers estimated the amino acid composition for the Last Universal Ancestor (LUA). This composition was depleted and enriched in several of Trifonov's modern and ancient amino acids, respectively. The Brooks and coworker set of ancient proteins contains two almost equal-sized subsets: RNA-associated proteins and enzymes. We found the RNA-associated proteins from the LUA to be much more extensively depleted in the modern amino acids than were the enzymes. We also noticed that the more ancient amino acids are predominantly disorder-promoting while the more modern amino acids are predominantly order-promoting. Two different disordered protein predictors suggest the RNA-associated proteins to be disordered and the enzymes to be structured, which agrees with laboratory experiments on the modern protein counterparts. If the RNA-associated proteins are representative of the proteins present in the earliest life forms, then these proteins lacked regular 3D structure. Therefore, we propose that: 1. the change from ancient to modern life forms depended on the evolution a protein disorder-to-structure transition, thus enabling the formation of protein enzymes; and 2. this evolutionary disorder-to-order transition was enabled by the introduction of the structure-promoting amino acids during the modernization of the genetic code.

1623-Pos Board B467

Large-scale Analysis of Thermo-stable, Mammalian Proteins Provides Insights into the Intrinsically Disordered Proteome

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Intrinsically disordered proteins (IDPs) are predicted to be highly abundant and play broad biological roles in eukaryotic cells, including signaling and regulation. However, these concepts are based on *in silico* analyses of whole genome sequences, not on large-scale proteomics analyses of living cells. Therefore, whether these concepts broadly apply to expressed proteins is currently unknown. Previous studies have shown that heat-treatment of cell extracts leads to partial enrichment of IDPs. Based on this, we sought to address the current dearth of knowledge about expressed IDPs by performing a large-scale proteomics study of thermo-stable proteins from mouse fibroblasts. Using a novel MudPIT strategy, we identified a total of 1,320 thermo-stable proteins from these cells and used bioinformatics methods to analyze their structural and biological properties. Interestingly, >900 of these expressed proteins were predicted to be IDPs. Unexpectedly, computational structural analyses revealed that, 1) disordered domains and coiled-coil domains occurred together in a large number of disordered proteins, suggesting functional interplay between these domains, and 2) >170 proteins contained lengthy domains (>300 residues) known to be folded. Reference to Gene Ontology Consortium functional annotations revealed that, while IDPs do, in fact, play diverse biological roles in mouse fibroblasts, they exhibit heightened involvement in particular functional categories, including, cytoskeletal structure and cell movement, metabolic and biosynthetic processes, organelle structure, cell division, gene transcription, and ribonucleoprotein complexes. We envision that these results not only reflect the specialized physiology of fibroblast cells, but also the general properties of the mouse intrinsically disordered proteome (IDP-ome). We will present these and our continuing studies of expressed, mouse IDPs, including, for example, analyses of the functional pathways associated with the over-represented functional categories noted above.
1

1624-Pos Board B468

A Robust Approach for Analyzing a Heterogeneous Structural Ensemble

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Intrinsically unstructured proteins (IUPs) are widespread in eukaryotes and participate in numerous cellular processes, but a structural explanation of the mechanisms they employ to recognize and bind their diverse targets has proved elusive. Transcriptional activator domains (TADS) are one class of IUPs that function by recruiting other factors into transcription complexes. TADS utilize electrostatic interactions to recognize binding partners, but it is unclear how an unstructured protein could perform this activity. To investigate this question, principal component analysis was performed on the atomic contact maps of an experimentally restrained ensemble of human p53TAD. This analysis permitted the identification of persistent structural features and their relative probabilities. Thirteen clusters of structures were identified that represented 98% of the ensemble. Potential surfaces of the aligned clusters showed the negative charges of the highly acidic p53TAD are uniformly organized on one face of the clusters. This observation provides a structural basis for the recruitment of other factors into transcription complexes and further supports the hypothesis that IUPs have evolved under selection to maintain specific structural features.

1625-Pos Board B469

Unfoldomics of Human Genetic Diseases

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Intrinsically disordered proteins lack stable structure under physiological conditions, yet carry out many crucial biological functions, especially functions associated with regulation, recognition, signaling and control. Recently, human genetic diseases and related genes were organized into a bipartite graph (Goh, K. I., Cusick, M. E., Valle, D., Childs, B., Vidal, M., and Barabasi, A. L. (2007) The human disease network. Proc Natl Acad Sci U S A 104, 8685-90). This diseaseome network revealed several significant features such as the common genetic origin of many diseases. We analyzed the abundance of intrinsic disorder in these diseaseome network proteins by means of several prediction algorithms, and we analyzed the functional repertoires of these proteins based on prior studies relating disorder to function. Our analyses revealed that (i) Intrinsic disorder is common in proteins associated with many human genetic diseases; (ii) Different disease classes vary in the IDP contents of their associated proteins; (iii) Molecular recognition features, which are relatively short loosely structured protein regions within mostly disordered sequences and which gain structure upon binding to partners, are common in the diseasome, and their abundance correlates with the intrinsic disorder level; (iv) Some disease classes have a significant fraction of genes affected by alternative splicing, and the alternatively spliced regions in the corresponding proteins are predicted to be highly disordered; and (v) Correlations were found among the various diseasome graph-related properties and intrinsic disorder. These observations provide the basis for the construction of the human-genetic-disease-associated unfoldome.

1626-Pos Board B470

Modeling the Unfolded States of Tau protein and p21(145-164)

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Intrinsically disordered proteins (IDPs) play essential roles in a number of normal and pathological processes, but unlike most other proteins they can adopt a variety of distinct conformations in solution. Here we propose a novel approach, called Energy-minima Mapping and Weighting (EMW), for constructing models of IDPs. The method samples energetically favorable conformations within an IDP and uses these structures to construct ensembles that are consistent with a given set of experimental data. A unique feature of the method is that it does not strive to generate a single ensemble that represents the unfolded state. Instead we construct a number of candidate ensembles, each of which agrees with a given set of experimental constraints (such as NMR chemical shifts and hydrodynamic radii and residual dipolar coupling constants) and focus our analysis on local structural features that are present in all of the independently generated ensembles. We apply the method to two natively unfolded proteins: tau protein, which plays a role in Alzheimer's Disease pathology, and ($p21^{145-164}$), a small IDP that binds to approximately 25 targets and is believed to play a role in cellular signaling. For tau protein, we deduce structural features that may explain the proclivity of tau mutants to form pathologic aggregates and in the case of p21, we demonstrate that the peptide's intrinsic